

## C-Glycosylxanthones in Diploid and Tissue Culture-induced Autotetraploid *Davallia fejeensis*

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1,3,6,7-tetrahydroxy-C-glycosylxanthones are phenolic compounds which have been found relatively infrequently in ferns. A recent survey (Wallace et al., 1982) reported their occurrence in seven genera: *Asplenium*, *Athyrium*, *Elaphoglossum*, *Cardiomanes*, *Hymenophyllum*, *Trichomanes*, and *Marsilea*. They have also been reported in *Ctenitis decomposita* (Bohm, 1975). The present communication reports C-glycosylxanthones in *Davallia fejeensis* Hooker. Diploid and tetraploid samples of both the sporophytic and gametophytic generations were examined.

### MATERIALS AND METHODS

Rhizome tips (about 2 cm long) of diploid *Davallia fejeensis* were collected in the Enid A. Haupt Conservatory of the New York Botanical Garden, cleaned, rinsed in tap water and further trimmed. Voucher specimens are at NY. Explants (6-8 mm long) were cultured on 1% agar-gelled sterile nutrient medium of Knudson as modified by Steeves et al. (1955) and supplemented with 2% sucrose in order to provide control diploid sporophytes growing in axenic culture. Roots, leaves, and rhizomes of the resulting plants were excised and induced to differentiate into aposporous diploid gametophytes. Stock cultures of diploid gametophytes were multiplied and further grown on liquid media supplemented with 0.6% agar to effect fertilization and raise tetraploid sporophytes. Tetraploid gametophytes were isolated from this tissue culture-induced tetraploid material by repeating the procedure followed at the diploid level. In this way, both diploid and tetraploid sporophytes and gametophytes were made available for this study.

Phenolic compounds were isolated by two-dimensional paper chromatography of an 80% methanol extract of green material in t-BuOH-HOAc-H<sub>2</sub>O, 3:1:1 (TBA) and 15% aqueous acetic acid (HOAc), followed by one-dimensional paper chromatography in water. C-glycosylxanthones appeared as orange compounds in ultraviolet light and turned yellow when fumed with ammonia. Both purified compounds were co-chromatographed with mangiferin and isomangiferin isolated from *Asplenium montanum* Willd. (Bozeman & Radford 11552, NY). R<sub>f</sub> values in TBA, BAW, HOAc, and H<sub>2</sub>O were: mangiferin, 0.31, 0.43, 0.43, 0.12; isomangiferin, 0.19, 0.29, 0.23, 0.04; *Asplenium* mangiferin, 0.34, 0.42, 0.42, 0.11; *Asplenium* isomangiferin, 0.21, 0.32, 0.23, 0.03; and rutin standard, 0.43, 0.45, 0.56, 0.25. The BAW and HOAc values are very similar to those of Smith and Harborne (1971), but the TBA and HOAc values differ from those of Markham and Wallace (1980). Absorption spectra (MeOH, nm) were: mangiferin, 232, 260, 270sh, 310, 364; isomangiferin, 240, 258, 270sh, 312, 365. The compounds were unaffected by acid hydrolysis (1 hr, 2N HCl, 100°C).

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## RESULTS AND DISCUSSION

The C-glycosylxanthenes mangiferin and isomangiferin were the only phenolic compounds appearing on the two-dimensional chromatograms of an 80% methanol extract of each sample. The diploid and tetraploid sporophytes contained relatively large amounts of both compounds, but in both cases the mangiferin spot was much larger than the isomangiferin spot. Gametophyte extracts contained very small amounts of each compound. Further identification was performed only on compounds from the sporophytes. Spots from the chromatograms were eluted and then chromatographed in water to purify them sufficiently for ultraviolet spectrophotometry and co-chromatographic testing. The purified compounds showed reduced  $R_f$  values in comparison to those measured on the two-dimensional chromatograms, strongly suggesting that  $R_f$  values based on such chromatograms may be misleading.

These results suggest that the same C-glycosylxanthenes are produced in both sporophytes and gametophytes. This is comparable to the only similar published study of fern flavonoids (Petersen & Fairbrothers, 1980), where the same flavonol glycosides appeared in both generations. Secondly, it appears that an induced doubling of the chromosome number in *Davallia* has no effect on the production of C-glycosylxanthenes in either generation. If we accept that the C-glycosylxanthenes in *Davallia* are equivalent to the flavonoids in flowering plants, then the effects of induced chromosome doubling on phenolic production in ferns may be compared with the effect of a similar chromosome doubling in angiosperms. Mears (1980) has reviewed the chemistry of polyploids. In some cases of induced autotetraploids in *Phlox* (Levin, 1968), no qualitative differences in phenolics were detected; in another study in the same genus (Levy, 1976), such differences did occur. In *Briza* (Murray & Williams, 1976) qualitative differences in phenolic production sometimes occurred in induced tetraploids. A more complex situation occurred in autotetraploids of *Gibasis* (del Pero de Martinez & Swain, 1977), where polyploidy is correlated with Robertsonian Fusion, a phenomenon which may be responsible for changes in phenolic expression.

This is the first study of the chemistry of induced autoploidy in fern gametophytes and sporophytes. The tissue culture method followed in this study is an excellent way to effect such changes in chromosome numbers. A survey for the presence of C-glycosylxanthenes in other fern genera is being undertaken in our laboratory; it is possible that these compounds may not be as rare as they were previously thought to be.

## LITERATURE CITED

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## REVIEW

✓ **THE GENUS SELAGINELLA IN TROPICAL SOUTH AMERICA**, by A. H. G. Alston, A. C. Jermy, and J. M. Rankin. *Bull. Brit. Mus. (Nat. Hist.), bot. ser.* 9(4):233-330. 1981. £14.00 postpaid.—A. H. G. Alston was the premier *Selaginella* authority in the middle of this century. He published extensively on the genus in the Old World and in southern South America, Brazil, the West Indies, and Central America in the New World. At the time of his death, he had begun to study *Selaginella* from the Andean countries and the Guyanas. The present paper completes and extends Alston's work and includes Brazil, thus accounting for virtually all of tropical South America. The region contains 133 species and six infraspecific taxa of *Selaginella*. A table of species distribution by countries shows that Colombia has by far the greatest number of species. The bracketed key to the species can be read forward or backward, a decided advantage because there are no species descriptions. Each taxon treated has a synonymy, a statement of range, a list of specimens, and, sometimes, additional notes. The authors have uncovered some overlooked synonyms in rare literature, always a pleasure to see. However, most synonyms state only the country where the type was collected, rather than the type locality, the collector and number, and the herbarium of deposit. A list of references and an index to accepted names and synonyms concludes the volume. New taxa are illustrated by a photograph of the herbarium sheet and by SEM photographs of the leaves, which range in magnification upwards from a mere eight times natural size, a useful technique for illustrating clearly the cilia and other small details of the leaves. This paper is indispensable for identifying *Selaginellas* from tropical America and will be the standard reference for many years to come.—D.B.L.